Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias

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Imatinib (STI571 or CGP57148B) is an innovative treatment for tumours with a constitutively activated form of *c-ABL, c-KIT,* or *PDGFR*. Such tumours include Philadelphia-chromosome-positive (Ph-positive) leukaemias, gastrointestinal stromal tumours, and PDGFR-positive leukaemias. Diseases such as primary hypereosinophilia and dermatofibrosarcoma protuberans also seem to respond to imatinib. Clinical trials assessing the therapeutic effects of imatinib have shown that the drug is highly effective with few associated side-effects, achieving durable cytogenetic responses in many patients with chronic-phase *BCR-ABL*-positive leukaemias. However, the emergence of resistance, particularly in patients with acute leukaemias, has prompted intense research, and many are concerned about the future prospects for imatinib. The resistance has been found in patients with acute-phase disease, but may also occur in patients with chronic-phase disease. Two cellular mechanisms for resistance to imatinib have been identified: amplification of *BCR-ABL* gene and mutations in the catalytic domain of the protein. In addition, suboptimum inhibition of BCR-ABL in vivo could contribute to the selection of resistant cells. We have summarised all currently available data on resistance to imatinib, both published and unpublished, including the mechanisms of resistance identified so far, and their clinical relevance to the different forms of Ph-positive leukaemias is discussed. Furthermore, we discuss strategies to overcome or prevent the development of resistance.

Lancet Oncol 2003; **4:** 75–85

Treatment options for patients with chronic myeloid leukaemia (CML) and other types of leukaemia positive for the Philadelphia chromosome are entering a new phase, owing to the development of imatinib mesylate (STI571).[1] For the first time, a specific and fairly non-toxic drug is able to induce durable remissions in most patients with Ph-positive leukaemias and cytogenetic responses in most patients with chronic-phase CML. However, because of the very brief follow-up period, a full assessment of this drug has not been possible. Longer follow-up studies of patients who are currently being treated with imatinib are essential, to investigate further the successes and failures of imatinib. This review discusses the mechanism of action of imatinib, the current status of clinical trials of the drug in the treatment of different leukaemias and solid tumours, and the cellular and pharmacokinetic factors that cause or favour the development of resistance to the drug.

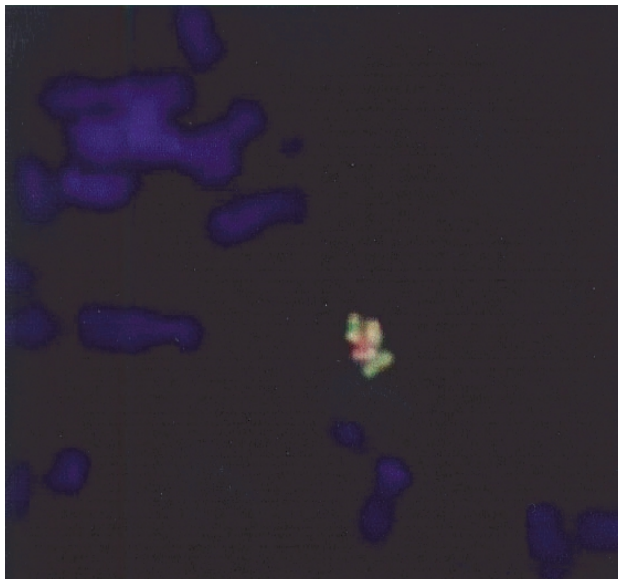

Figure 1. FISH analysis of LAM84R cells. A metaphase sample of LAMA84R cells shows a marker chromosome highly labelled with a BCR-ABL-specific probe, indicative of gene amplification.

Structure and mechanism of action

Imatinib belongs to a class of compounds known as the 2-phenylaminopyrimidines, which were developed at Ciba Geigy (Novartis) in the early 1990s.[2,3] The drug is a potent inhibitor of three tyrosine kinases: Ableson (ABL), platelet-derived growth-factor receptor (PDGFR) α and β, and KIT. The Abelson-related gene (*ARG*) is also inhibited by imatinib.[4] The inhibitory action of imatinib against ABL was the feature that prompted its clinical development.

ABL (located on chromosome 9q34) becomes fused to the *BCR* gene (located on chromosome 22q), as a result of a chromosomal translocation (figure 1). The mutated chromosome 22—the Philadelphia chromosome (Ph)—is the cause of almost all cases of CML, 20–30% of adult acute lymphoblastic leukaemia (ALL), and about 5% of ALL in children. About 2% of acute myeloid leukaemias (AML) are also Ph positive. The hybrid gene encodes the oncogenic

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fusion protein BCR-ABL, which transforms bone-marrow stem cells through its deregulated tyrosine kinase activity;[5] the deregulation is due to loss of a regulatory domain (normally at the 5' end of the *ABL* gene) in the hybrid gene.[6]

Therefore, in Ph-positive leukaemia, imatinib is a rational strategy aimed at inhibiting the mechanism by which neoplastic transformation occurs.[7,8] In *BCR-ABL*-expressing cells, imatinib produces three related effects: inhibition of BCR-ABL autophosphorylation and substrate phosphorylation, inhibition of proliferation, and induction of apoptosis.[9–11] No major induction of differentiation has been consistently noted, although synthesis of haemoglobin (a marker of erythroid differentiation) in K562 cells has been observed after treatment with imatinib.[9] The effects of imatinib have different kinetic profiles: inhibition of phosphorylation can be observed 1 min after imatinib treatment,[10] whereas blockage of proliferation and induction of apoptosis require longer (minimum 16–20 h) inhibition of BCR-ABL.[10,12] Furthermore, the kinetics vary among different cell types: cells from patients with acute leukaemia (Ph-positive ALL or blast-crisis CML) or cell lines derived from patients with acute leukaemia, have faster kinetic profiles than cells from patients with chronic-phase CML in whom induction of apoptosis can take as long as 100 h.[10] The faster kinetics of induction of apoptosis in acute leukaemia cells may be a reflection of many additional abnormalities which, in the absence of BCR-ABL activity, could encourage proapoptotic pathways.

Imatinib was originally thought to inhibit kinase activity by acting as a competitive inhibitor of the ATP-binding pocket. However, recent structural resolution of the ABL catalytic domain complexed with an imatinib homologue, showed that the drug occupies only part of the ATP-binding pocket of the enzyme, and that it produces its inhibitory effects by binding to and stabilising the inactive, non-ATP-binding form of BCR-ABL.[13] Definition of the mechanism of action of imatinib has been particularly important in elucidation of some mechanisms of resistance.

Current status of clinical trials of imatinib

Imatinib has been under clinical investigation for almost 4 years. During this time more than 20 000 patients, most with CML or Ph-positive ALL, have received the drug.

Acute leukaemia is defined as more than 30% blast cells in the bone marrow or peripheral blood. Patients in this category present with de novo acute leukaemias (Ph-positive ALL or AML) or blast-crisis CML. Patients with acute leukaemia showed a high (more than 50%) rate of initial haematological response. 52% of patients with myeloid-blast-crisis CML and 60% of patients with Ph-positive ALL or lymphoid-blast-crisis CML showed a reduction in blast values corresponding to a haematological response.[14,15] Unfortunately, the therapeutic effects of imatinib in such patients have been short-lived (3–6 months), and resistance to the drug and relapse occurs quickly in almost all patients.[14,15] Although patients affected by myeloid-blast-crisis CML seem to respond slightly better than patients with ALL or lymphoid blast-crisis CML, at present, overall, imatinib in this category of patients can be considered a qualified failure.

In patients with accelerated-phase CML (15–30% blast cells, more than 20% basophils, or platelet counts less than $1x10^{11}$/L) durable major cytogenetic responses can be achieved, but only in about 25% of patients; however, prolonged survival can probably be achieved even in the absence of a cytogenetic response.[16]

Patients with chronic-phase CML (more than 15% blast cells, less than 20% basophils, and a platelet count of more than $1x10^{11}$/L) who have disease that is intolerant, resistant, or refractory to interferon treatment, respond better than patients who have more advanced disease; major cytogenetic responses were achieved in 64% of patients.[17] Although these responses seem to be durable,[17] most patients with complete cytogenetic responses are still positive for the BCR-ABL fusion protein when assessed by quantitative PCR[18] or reverse transcriptase PCR, even after 2 years of treatment (Gambacorti-Passerini, unpublished data). Overt clinical resistance to imatinib is rarely observed in this group of patients. However, the issue of resistance, although of immediate relevance in patients with advanced disease, is also potentially relevant in patients with chronic-phase disease. Important clinical responses were also obtained in patients with malignant disorders caused by structural alterations of the *PDGFRβ* gene.[19,20]

Imatinib is also active against a rare form of non-epithelial gastrointestinal cancer called gastrointestinal stromal tumours (GISTs), many of which have activating mutations of the *c-KIT* gene, another target of imatinib. In this group of patients, objective response rates of 53% and 54% were obtained,[21,22] with therapeutic effects reported within a few days of treatment in many cases. These responses seem to be durable and linked to *c-KIT* mutations in the tumour; risk of progression after imatinib treatment was eight times higher in patients without *c-KIT* mutations than in patients with *c-KIT* mutations (44% *vs* 5·8%).[23] Thus, resistance to imatinib seems to arise from diverse molecular pathogenetic mechanisms present in some GISTs, with some tumours resulting from *c-KIT* mutations and other tumours from different and unknown pathogenetic mechanisms. Imatinib also shows activity against primary hypereosinophilia[24,25] and in dermatofibrosarcoma protuberans.[26,27]

Definition of resistance to imatinib

Various definitions of resistance have been used in published studies, with various associated factors (eg, cytogenetic, haematological, and refractory status). We have defined haematological resistance to imatinib as the lack of a complete haematological response in patients with chronic-phase disease,[17] a return to chronic phase for patients in acute phase,[16] or a partial response in patients with blast-crisis CML or Ph-positive ALL.[15] Resistance can be primary (no response to imatinib after the initial treatment) or secondary (development of resistance after achieving an objective response).

Clinical resistance to imatinib is common in patients with acute leukaemia (blast-crisis CML or Ph-positive ALL), and occurs within 3–6 months of treatment in over 70% of patients.[14,15] Once resistance develops, the disease is rapid and aggressive in most patients.

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By contrast, patients in chronic-phase disease respond favourably to imatinib. Over 50% of these patients (64% at 26 months)[17] experience a major (generally complete) cytogenetic response, in many within the first 3–6 months of treatment. Such responses have so far proved durable.[17] However, most patients remain positive for BCR-ABL when tested with PCR, which suggests that Ph-positive leukaemic cells persist in 10^{-2} to 10^{-5} cells. In a few patients with chronic-phase disease, relapse developed after a complete cytogenetic response was achieved—although this was rare.[28] Whether the mechanisms of resistance in these patients are the same as those for patients with acute leukaemia remains to be seen. Therefore, although 64% of patients with chronic-phase disease now show durable major cytogenetic responses more than 2 years after starting imatinib therapy, with 49% of them showing complete cytogenetic responses,[17] the development of resistance remains a potentially devastating problem in this group of patients.

Molecular mechanisms of resistance to imatinib

Since imatinib inhibits the enzymatic activity of BCR-ABL, the measurement of BCR-ABL tyrosine kinase activity is the best way to assess the degree of activity of the drug. BCR-ABL activity can be measured with an enzymatic assay, or by analysis of the tyrosine-phosphorylation status of BCR-ABL (autophosphorylation) or its substrates,[10] such as the adaptor protein CRKL.[29] The enzymatic assay requires the immunoprecipitation of BCR-ABL and therefore is seldom used to monitor patients. However, the analysis of BCR-ABL autophosphorylation or of CRKL phosphorylation has been used in several studies. Inhibition of BCR-ABL activity was evident in patients with chronic-phase disease or acute leukaemia within 24–72 h of imatinib treatment.[29,30] However, inhibition was not complete (generally about 50%) and was substantially lower than that obtained in vitro with a concentration of imatinib equivalent to that detected in the blood of the patients.[30] This finding is an important factor in elucidation of resistance.

At the time of relapse, patients typically show the reappearance of a fully active BCR-ABL protein.[30] This observation has been confirmed by several[31] but not all groups (Hochhaus A, et al, unpublished data). If confirmed, these findings would indicate that the leukaemic cells remain dependent on the transforming activity of BCR-ABL. Resistance, therefore, would develop because cells were able to maintain BCR-ABL in an active form despite treatment with imatinib, rather than becoming independent of BCR-ABL-mediated signalling. This point emphasises the importance of targeting early events in the transformation process

Resistance develops as cells find ways to maintain sufficient amounts of BCR-ABL signalling; three different mechanisms by which this result is achieved are *BCR-ABL* gene amplification, *BCR-ABL* gene mutation, and incomplete BCR-ABL inhibition, which could be involved in favouring the selection of resistant cells.

BCR-ABL gene amplification

The amplification of the *BCR-ABL* gene in imatinib-resistant leukaemic cells was initially described in a LAMA84R cell line generated by culturing cells in increasing concentrations of imatinib.[32] A crucial factor in this selection process was the use of marginally active concentrations of imatinib in the initial culture (less than the IC_{50} ie, the concentration leading to 50% inhibition). Furthermore, culture of cells at concentrations equal to or above the IC_{50} did not permit the selection of resistant cells, owing to the massive induction of apoptosis. LAMA84R cells can be grown in 0·7 μmol/L imatinib, and have an IC_{50} value of 1 μmol/L, compared with 0·1 μmol/L for the LAMA84 cell line from which the cells were derived. LAMA84R cells express about five times more BCR-ABL protein than the parent LAMA84 cells. The protein, however, maintains an intrinsic sensitivity to imatinib. Further analysis showed that the mechanism causing BCR-ABL overexpression was the amplification of the *BCR-ABL* gene, measured by a marker chromosome, highly labelled by a *BCR-ABL*-specific probe and containing about 15 copies of the hybrid gene (figure 1). The analysis of other cell lines resistant to imatinib confirms the existence of this mechanism, as well as the lack of *BCR-ABL* mutations, although some differences were noted among the cell lines used.[33,34]

Table 1. Presence of *BCR-ABL* mutations and amplifications in patients with Ph-positive leukaemias resistant to imatinib

Ref	Primary resistance (refractory)				Secondary resistance							
					BCR-ABL mutation				*BCR-ABL* amplification			
	M	L	U	Total	M	L	U	Total	M	L	U	Total
35	..	..	..	..	3/6	3/3	..	6/9	2/6	1/3		3/9
36	..	..	..	..	2/4	5/5	4/9	11/18	..	..	1/18	1/18
37, 60	..	0/9	..	0/9	..	7/9	..	7/9	..	0/15	..	0/15
38	0/17	0/1	0/6	0/24	9/17	0/1	..	9/18	8/26*	..	..	8/26
41	..	..	..	..	..	7/7	0/1	7/8	..	..	..	
42	..	..	..	..	13/20	4/5	3/6	20/31	..	..	..	
Unpublished data‡	..	..	..	..	..				2/13	1/6	2/10	5/29
Total (%)	**0/17** (0)	**0/10** (0)	**0/6** (0)	**0/33** (0)	**27/47** (57)	**26/30** (87)	**7/16** (44)	**60/93†** (64)	**12/45** (27)	**2/24** (8)	**3/28** (11)	**17/97** (18)

M, myeloid; L, lymphoid; U, unidentified (patients who developed resistance while in chronic or accelerated phase and for whom phenotypic analysis could not be done). *Six patients presented Ph duplications, two showed more than two copies of *BCR-ABL*.†At least 38 of 60 cases showed mutations at codon 255 (20 cases) or 315 (18 cases). ‡Gambacorti-Passerini.

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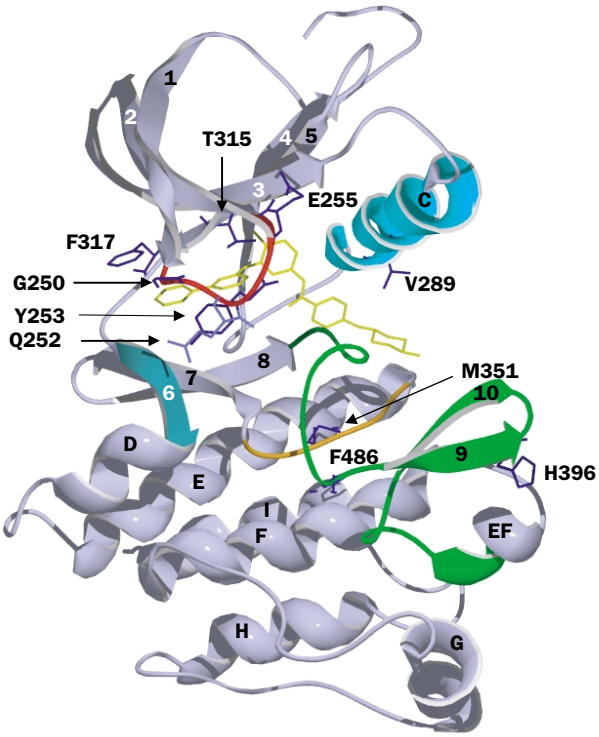

Figure 2. ABL in complex with imatinib (yellow). The location of mutations are highlighted along with the activation loop (green), nucleotide-binding loop (red), and catalytic loop (orange). β strands are numbered and α helices are lettered according to the nomenclature used for insulin-receptor tyrosine kinase.[47,49] The region including N322 and interacting with the nucleoside-binding loop is shown in cyan.

BCR-ABL gene amplification has subsequently been observed in clinical samples obtained from patients who are resistant to imatinib, with an overall frequency of about 18% (table 1).[35–38] In some patients with resistant disease, *BCR-ABL* seems to be overexpressed in the absence of gene amplification,[38] pointing to the existence of other mechanisms, such as changes in the transcriptional, translational, or post-translational control of BCR-ABL concentrations.

Although overexpression of *BCR-ABL* can confer resistance to imatinib, increased expression of the protein has been reported to be toxic to cells when imatinib is abruptly withdrawn;[39] the clinical relevance of this property remains untested. The identification of *BCR-ABL* amplification as a mechanism of resistance to imatinib shows that there is great dependence on BCR-ABL-mediated signalling even in advanced leukaemia. Because imatinib produces a decrease in the degree of signalling resulting from a given amount of protein, leukaemic cells expressing increased amounts of the targeted protein will be selected. These resistant cells maintain the minimum amount of signal transduction activity needed for cell survival, even in the presence of imatinib.

BCR-ABL mutations

Because imatinib was originally thought to be a competitive inhibitor of the ATP-binding site of ABL, mutations affecting imatinib binding were judged likely to produce non-functioning (kinase dead) enzymes. However, later studies showed that the docking of an imatinib analogue inside ABL is only partly superimposable over that of ATP.[13] Although such experiments were done with an imatinib analogue, similar data are also available for crystals of ABL complexed with imatinib (figure 2).[40] The phenylamino-pyrimidine part of imatinib interacts with M318, in a similar way to the interaction with ATP, but it then diverges from ATP by an angle of about 90°. In addition, imatinib is now known to bind and stabilise ABL in its inactive conformation (in which no ATP binds to the kinase) rather than competing with ATP for binding. Therefore, mutations in the kinase domain of BCR-ABL may be able to disrupt imatinib binding without interfering much with its enzymatic activity.

Several groups have succeeded in identifying at least ten different mutations in leukaemic cells from patients who had developed resistance to imatinib.[35–38,41,42] Mutations have been described at positions 250, 252, 253, 255, 289, 315, 317, 351, 396, and 486. The two most frequently occurring mutations were those affecting aminoacids E255 and T315 (table 1); at position 255, glutamine is substituted by either a lysine (E255K)[43] or a valine (E255V),[44] and at position 315, threonine is substituted by an isoleucine residue.[35] Although some mutations have been well characterised in terms of mechanism of action, or experimentally validated in terms of IC_{50},[35,41] others have been observed only in cells from resistant patients, and their relation to resistance needs further investigation. Most mutations have been detected in patients with acute leukaemia but some were also detected in patients

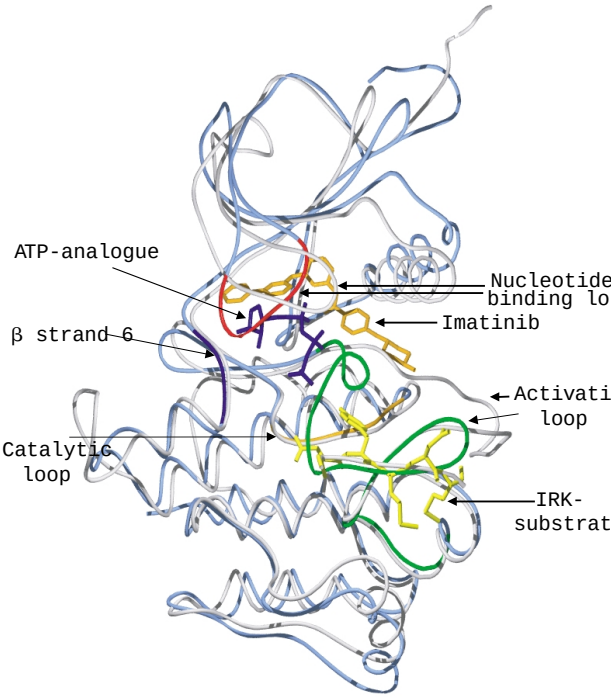

Figure 3. The C-terminal lobes of the inactive conformation of ABL[13] and the active conformation of its homologue, the insulin receptor tyrosine kinase (IRK3P).[47] ABL and IRK3P are in cyan and grey respectively. The nucleotide-binding loop of ABL is shown in red. The conserved region interacting with the nucleoside-binding loop is labelled as β strand 6.

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with chronic-phase CML who became resistant to imatinib, most without achieving a complete cytogenetic response.[38] These mutations were not observed in samples obtained before imatinib treatment. Since mutations can be identified only when mutated cells account for at least 10% of the overall cell population, these data indicate that mutant cells were present at low prevalence before imatinib therapy. This idea is not surprising given that these mutations do not confer any growth advantage in the absence of imatinib. The short time required for the emergence of resistance suggests that mutants were present before treatment and were selected only because of the specific pressure exerted by imatinib. The study by Roche-Lestienne and colleagues, who used a sensitive technique called allele specific oligonucleotide-PCR to detect mutations, supports such a conclusion.[45] These data also indicate the extreme degree of heterogeneity of Ph-positive acute leukaemias; clones harbouring mutations probably exist in almost all genes and so could be selected if a specific pressure were applied.

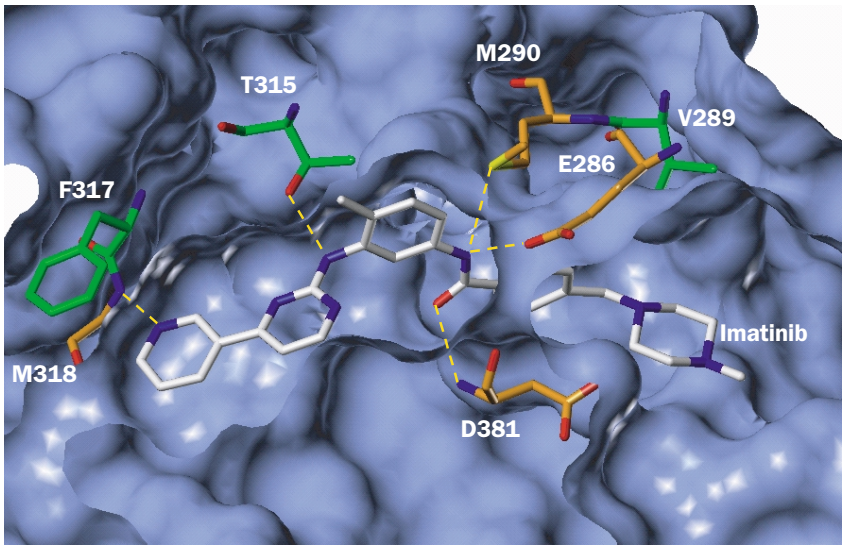

Figure 4. Mutations affecting the imatinib-binding site in ABL. Imatinib, the aminoacids involved in direct hydrogen bonding, and the aminoacids involved in some mutations are shown as colour coded stick models. Nitrogen atoms are shown in blue, oxygen atoms in red, and sulphur atoms in yellow. The carbon atoms of the mutational positions are in green, those of imatinib are in white, and those of the remaining aminoacids are in orange. The molecular surface (blue) represents the van der Waals interactions between imatinib and the protein. Hydrogen bonds are represented by dashed yellow lines. For clarity, the nucleotide-binding loop has been omitted.

Mutations have not been found in patients who did not initially respond to imatinib (primary resistance, table 1). Thus, there are mechanisms other than mutations that can lead to the development of resistance. In these patients, imatinib was unable to exert a selective pressure because it could not eliminate cells.

BCR-ABL mutations also seem to be significantly more frequent in patients with lymphoid leukaemia (ALL, lymphoid blast-crisis CML) than in patients with myeloid leukaemia (table 1; 26 of 30 [87%] versus 27 of 47 [57%], p=0·01).

The two identified mechanisms of resistance to imatinib (*BCR-ABL* mutations and amplification) probably account for almost all cases of resistance in patients with lymphoid leukaemia, whereas additional mechanisms such as mutations outside the ABL catalytic domain or changes in imatinib cellular-transport mechanisms[34] could be present in some patients with myeloid leukaemias and in patients who do not initially respond to imatinib.

Structural considerations of ABL mutations in patients resistant to imatinib

Studies done during the past year have shown that the three-dimensional distribution of known mutations within the ABL structure (figures 2 and 3) cluster into four main groups. One group of mutations (V289A, T315I, F317L,[35,36] and Gambacorti-Passerini, unpublished data) can be found in the imatinib-binding site and directly interact with the inhibitor via a hydrogen bond (T315) and Van der Waals' interactions (V289, T315, and F317; figure 4). A second group (Y253F, G250E, E255K, and Q252H) includes aminoacids that form the nucleotide-binding loop for ATP (also known as the first loop or p loop).[36,41,44,46] The side chain of Y253 forms Van der Waals interactions with imatinib, whereas the aminoacids E255, G250, and Q252 do not interact directly with the inhibitor (figure 5). The third group of mutations is located in the activation loop and close to the hinge region of the flexible sequence (figure 6). So far a single mutation at position 396 (H396P) has been described in this region.[41] Lastly, M351T and F486S mutations include aminoacids distant from the imatinib-binding site, which form a hydrophobic patch between helices E, F, and I in the C-terminal lobe of the enzyme, which is highly conserved within the tyrosine kinase family (figure 7).[36,38,42,47]

Despite their different locations, several of these mutations decrease the binding affinity of BCR-ABL for imatinib,[35,38,41] although with different indices of resistance.[41] In addition, kinase activity is not abrogated by the mutations, although some mutants have enzymatic activity lower than that of wild-type BCR-ABL.

These findings raise two main questions: what is the mechanism of decreased binding affinity between BCR-ABL and imatinib in each group of mutations, and do these mechanisms have something in common?

The mechanism by which mutations in the imatinib-binding site lead to a reduced binding affinity can be easily explained by looking at the snug fit of the inhibitor in the site (figure 4), which does not really permit any modification of the binding site without compromising binding affinity.[13] If T315 is mutated to isoleucine, a hydrogen bond is lost and the binding site becomes too narrow to accommodate the

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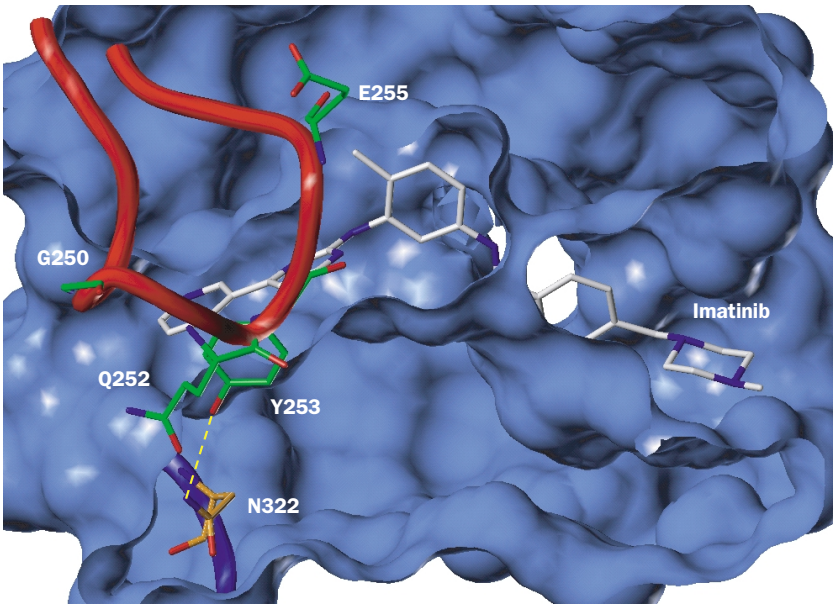

Figure 5. Mutations affecting the nucleotide-binding loop (red). Nitrogen atoms are shown in blue and oxygen atoms in red. The carbon atoms of the mutational positions and imatinib are in green and white respectively, and those of non-mutated residue N322 are in orange. The molecular surface (blue) represents the van der Waals interactions between imatinib and the protein. The hydrogen bond between Y253 and N322 of β-strand 6 is shown as a broken yellow line.

phenylamino moiety.[13,35] The F317L and V289A mutations cause a loss of Van der Waals contacts with the pyridine and piperazyl moiety of the inhibitor, respectively.[36] These aminoacids are located at the two extremes of the binding site and shield it from water, thus the reduction in the size of the aminoacid side chain increases the accessibility of water to the binding site and the strength of the interaction within the binding site is reduced because of increased competition by water.

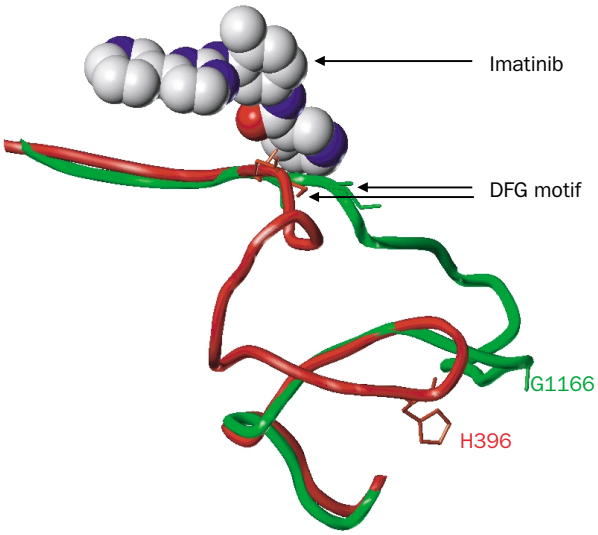

Figure 6. The activation loop of ABL[13] compared with the active conformation of IRK (IRK3P).[47] The gate-closed conformation of ABL is shown in red and the gate-open conformation of IRK3P is shown in green. Aminoacid (H396) affected by mutation in ABL is situated at the end of the activation loop, and the corresponding aminoacid (G1166) of IRK3P is shown in green.

To explain the effects of the mutations located elsewhere, other issues have to be considered, such as the kinase mechanism and its thermodynamic properties. For this reason, ABL (inactive conformation) has been compared with the highly homologous (76%) insulin-receptor tyrosine kinase (IRK, active conformation; figure 3). All tyrosine kinases cycle between an active conformation (activation loop phosphorylated and open) and an inactive conformation (activation loop non-phosphorylated and closed). Structural and thermodynamic data on wild-type IRK, and a mutant form D1161A, are available.[47–49] The D1161A mutation causes loss of hydrogen bonds, which link the activation loop to other regions of the terminal lobe that are structurally conserved.[49]

Several observations can be made on the basis of this knowledge. First, every catalytic cycle of the tyrosine kinase comprises binding of ATP, conformational changes, and a chemical reaction. In the presence of millimolar concentrations of ATP (similar to the concentration within cells), the inactive conformation (with the activation loop in the gate-closed position) is in equilibrium with the active conformation (with the activation loop in the gate-open position; figures 3 and 6).[49] This equilibrium is regulated by the energy differences between the two conformations. The analysis of the conformational free energy revealed that the wild-type inactive conformation (conformational free- energy, -14·3 kcal/mol) is more stable than the wild-type active conformation (-10·2 kcal/mol), and D1161A has an intermediate value (-11·3 kcal/mol).[49] Second, the activation loop of D1161A assumes an intermediate conformation between the inactive and active conformation.[49] The degree of N-terminal to C-terminal lobe closure of the IRK mutant is also intermediate. Therefore, the difference in energies perfectly associates with changes in conformation, and is reflected by catalytic activity; the rate constant of the wild-type inactive enzyme is 50-times lower than that for the mutant enzyme.[49] Third, despite the major overall stability of the inactive conformation, the activation loop is in a less stable position in the inactive conformation than in the active form.[49] Such differences in the stability of a polypeptide domain within a protein, and its dependence on the conformational state, have been observed in other kinases undergoing conformational change.[50] The greater stability of the activation loop in the active conformation is associated with the formation of two additional β-sheets and interactions with the C-terminal lobe (figure 7).[47] Fourth, in addition to the activation loop, the nucleotide-binding loop has high intrinsic flexibility and undergoes conformational changes[13] that influence N-terminal to C-terminal lobe closure via the activation loop.[47] Finally, any modification of

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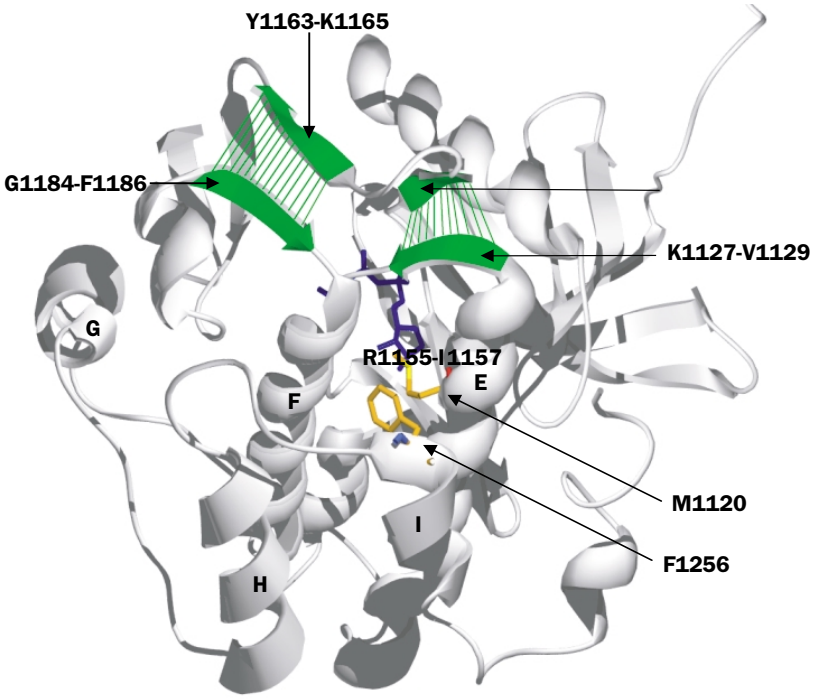

Figure 7. The C-terminal lobe of IRK3P with two new antiparallel β sheets (green) owing to phosphorylation of the activation loop.[47] Aminoacids Y1163–K1165 and G1184–F1186 of IRK3P correspond in ABL to Y393–A395 and N414–F416. Aminoacids R1155–I1157 and K1127–V1129 of IRK3P correspond in ABL to R386–M388 and N358–I360. Aminoacids M1120 and F1256, corresponding to the positions of ABL involved in the mutations (M351 and F486), are shown. Adenyl imido-diphosphate is shown in blue.

the ABL tyrosine-kinase domain could compromise the binding affinity of imatinib because of the snug fit of the inhibitor into its binding site.[13]

From these observations and the fact that the binding affinity of imatinib for the phosphorylated active form of ABL is much lower (7 μmol/L) than that for the non-phosphorylated form (38 nmol/L),[35] we can hypothesise the mechanisms leading to resistance in the various mutants in which the mutations are located on the nucleotide-binding loop, the activation loop, and the hydrophobic patch at the C-terminal lobe.

In the nucleotide-binding loop, the mutation Y253H or Y253F causes the loss of a hydrogen bond with N322 (figure 5); there is also a small reduction in hydrophobic interactions, because the histidine side chain is smaller than the tyrosine side chain. The loss of a hydrogen bond is probably the major contributing factor to resistance, because it causes a change in the equilibrium between the inactive and active conformation, forcing the balance towards the active conformation. From a structural point of view, the G250E mutation reduces the space available at the "entrance" of the imatinib-binding pocket, close to the pyridine moiety of the inhibitor (figures 2 and 5), thus access to the imatinib-binding pocket is hindered. However, since we do not know how the inhibitor enters the binding pocket, this explanation for resistance remains hypothetical. The link between the mutations, Q252H and E255K, and resistance cannot be explained structurally because the aminoacid side chains point towards the solvent. However, in terms of kinase activity, mutations in the nucleotide-binding loop probably change the protein dynamic towards activation by modifying the intrinsic flexibility of both the nucleotide-binding loop and the activation loop; thus the ability of the inhibitor to fit in the binding pocket is hindered (Gambacorti-Passerini, unpublished data).

The effect of mutations in the activation loop on conformational stability is shown in the studies of IRK D1161A, which has a mutation in the activation loop. The introduction of a proline at position 396 within the turn between β strands 9 and 10 (figure 6) of the inactive conformation is very unfavourable. This situation arises because the intrinsic steric constraints of the cyclic sidechain of proline favours the conformation of the activation loop in the active form; proline would be part of a β strand with an extended conformation (figures 3 and 6). Taking these structural considerations into account, we can hypothesise that resistance is caused by the equilibrium shift between the inactive and active conformations, with the consequent changes in conformation analogous to that observed for D1161A.

Finally, the introduction of polar residues (threonine or serine) into the hydrophobic patch (figures 2 and 7), as in the mutations M351T and F486S, decreases the strength of the hydrophobic interactions and thus decreases the stability of the structure. The inactive and active conformations could be affected differentially by this decreased stability. The structure of ABL in complex with imatinib (figure 2) and that of the active IRK (figure 7)[47] indicate that the hydrophobic patch is very important for the stability of the C-terminal lobe in the inactive conformation because it is the only structural element for packing helices E, F, and I together. By contrast, the active conformation of the enzyme is further stabilised in the C-terminal lobe by the two newly formed β sheets and the Van der Waals' interactions of their sidechains with the rest of the C-terminal lobe elements (figure 7). Such mutations are therefore likely to affect the conformational free-energy of the inactive conformation more than that of the active one. Thus, we can also hypothesise that there is an equilibrium shift in favour of the active conformation resulting in a decreased binding affinity for imatinib. This interpretation has been confirmed by recent experimental data.[51,52]

These structural factors suggest that the modification of the equilibrium between the inactive and active conformations due to mutations distant from the binding site could be a common mechanism for drug resistance; the stability of the active form would remain unaffected whereas the stability of the inactive conformation would decrease.

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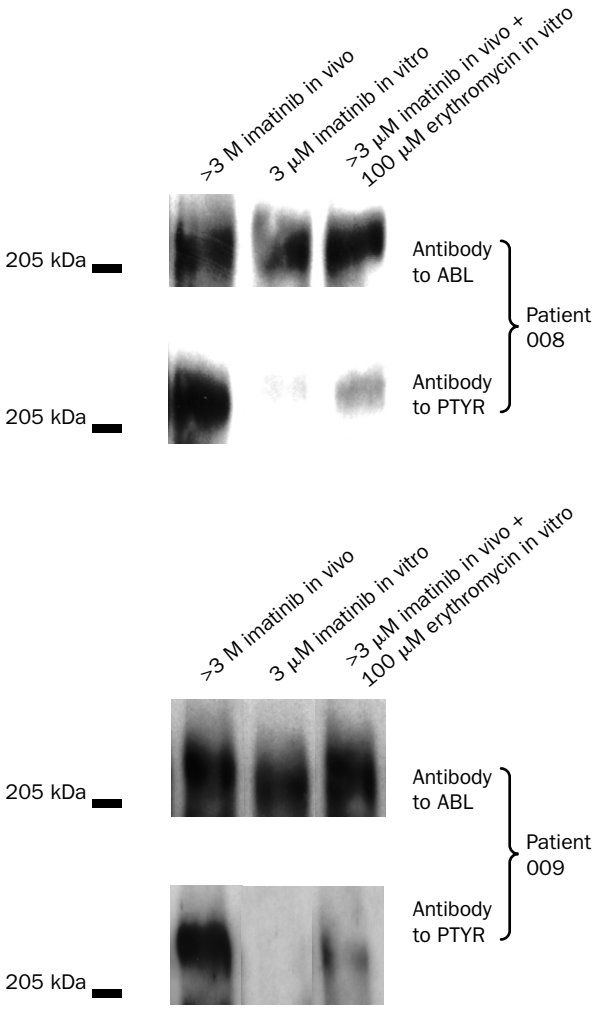
>3 M imatinib in vivo
3 μM imatinib in vitro
>3 μM imatinib in vivo + 100 μM erythromycin in vitro
205 kDa
Antibody to ABL
Patient 008
205 kDa
Antibody to PTYR
>3 M imatinib in vivo
3 μM imatinib in vitro
>3 μM imatinib in vivo + 100 μM erythromycin in vitro
205 kDa
Antibody to ABL
Patient 009
205 kDa
Antibody to PTYR

Figure 8. Effect of erythromycin on BCR-ABL phosphorylation in two patients resistant to imatinib. Unseparated blood samples were incubated with erythromycin (100 μmol/L) at 37°C for 1 h. Mononuclear cells were then separated, lysed, subjected to sodium dodecyl sulphate polyacrylamide-gel electrophoresis, transferred, and hybridised with anti-ABL or antiphosphotyrosine monoclonal antibodies. Controls include blood samples incubated with phosphate buffered solution and mononuclear cells incubated in vitro with 3 μmol/L imatinib after Ficoll separation. Reproduced from Blood Cells Mol Dis 2002; ***28:*** *361–72 with permission from Elsevier Science.*

Intracellular concentrations of imatinib

Pharmacokinetic data indicate that patients receiving 400 mg imatinib per day have plasma concentrations (steady-state values) constantly higher than 1 μmol/L.[53,54] In patients receiving higher doses (600–800 mg per day), increased plasma concentrations have been observed, with some patients having concentrations higher than 20 μmol/L. Since the IC_{50} value for BCR-ABL is 0·1–0·3 μmol/L, these findings show that these doses are more than sufficient to block the activity of the enzyme, at least in the non-mutated forms. However, there are experimental data that contradict this hypothesis.[30,55–58]

When leukaemic cells from patients were analysed after initial treatment with imatinib, only partial inhibition (around 50%) was observed,[29,30] although BCR-ABL could be completely blocked when the same cells were exposed in vitro to imatinib concentrations equivalent to the plasma concentrations.

Moreover, when leukaemic cells from three patients who had relapsed and had disease that was resistant to imatinib were assessed in vitro for sensitivity to imatinib, IC_{50} values of 0·1–0·3 μmol/L were obtained. However, despite plasma concentrations of imatinib greater than 3 μmol/L[30] and a complete block of BCR-ABL autophosphorylation and induced apoptosis after in vitro incubation with 3 μmol/L of imatinib, these patients still developed resistance.

Binding of α-1-acid glycoprotein (AGP), an acute-phase plasma protein, can inhibit the activity of imatinib.[57–59] Therefore, imatinib present in the plasma could be mostly bound to AGP and thus biologically inactive. To test this hypothesis, blood samples from two patients who were resistant to imatinib were incubated with erythromycin (which competes with imatinib for binding of AGP) for 1 h before separation of mononuclear cells and lysis. The activity of imatinib on BCR-ABL autophosphorylation was restored (figure 8), indicating that most plasma imatinib is bound to AGP so not active.

Furthermore, when patients who were being treated with imatinib received a simultaneous infusion of clindamycin, another molecule known to bind AGP with an affinity similar to erythromycin, large decreases in total plasma concentrations of imatinib were observed within minutes (figure 9);[53,55] this finding also shows that most imatinib present in plasma is not in equilibrium with tissues, and that there is a gradient between plasma and tissues.

Although the exact concentration of imatinib inside cells—the final target of the drug—is not known, these findings show that plasma concentrations of the drug are not a reliable indicator of the concentrations present inside leukaemic cells.

So what are the imatinib concentrations inside leukaemic cells, the final targets of the treatment? If the free plasma concentration of imatinib (total plasma concentrations divided by the percentage of free imatinib) is used to estimate the amount of the drug that can move between cells, tissue, and plasma, a rather low value—below 0·1 mg/L—is to be expected in cells. However, the free concentration is probably an underestimation of cellular concentrations of imatinib, since proteins other than AGP, such as albumin can bind imatinib, thereby reducing its free fraction, but without compromising its biological activity.[59] Therefore, concentrations of imatinib inside cells could lie anywhere between the free plasma and the total plasma concentrations as measured after clindamycin infusion. Since tissue concentrations of imatinib are substantially less than the concentrations detected in plasma, higher doses of imatinib for patients with acute leukaemia could be considered. However, higher doses might be counteracted by mutations in the catalytic domain of BCR-ABL—reported in a proportion of patients who are resistant to imatinib— which abolish or substantially decrease the binding of imatinib to its target. Although the development of imatinib analogues would be needed to overcome such mutations (if feasible

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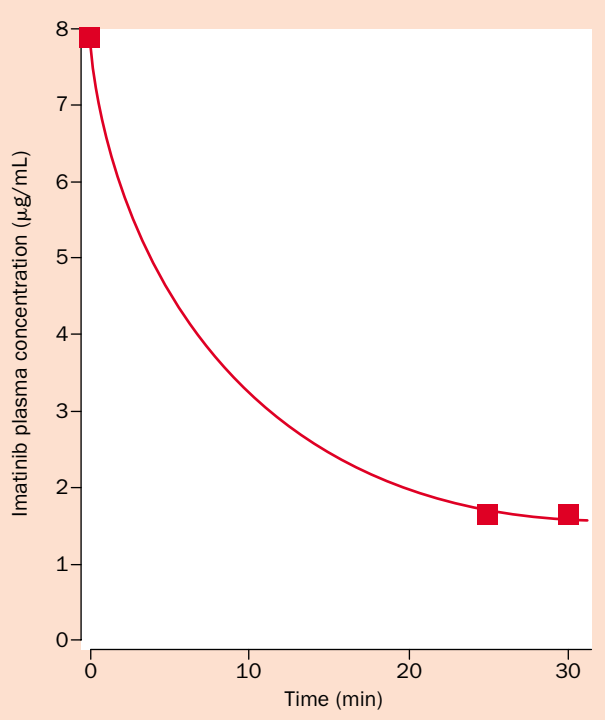

Figure 9. Effect of clindamycin (900 mg intravenously over 20 min) on imatinib plasma concentrations. One patient (number 008) at steady state received an intravenous infusion of clindamycin 23 h after the last dose of imatinib. 5 min and 10 min after the end of clindamycin infusion, blood samples were taken and total imatinib concentrations assessed. Adapted from ref 53.

given the high number of different mutants), the experimental conditions, in which a selective pressure is applied, can either favour or hinder the selection of resistant cells. The selection in vitro of LAMA84R cells (in which the BCR-ABL gene is amplified) was possible only when cells were exposed to marginally active concentrations of imatinib (below its IC_{50}) which were then gradually increased; use of active concentrations (0·3 μmol/L) from the start killed all cells, and none were selected. Therefore, exposure of leukaemic cells to marginally active imatinib concentrations, as probably occurs with doses used at present, will favour such a selection, and thus resistance.

Conclusion

It is less than 5 years since the first patients were treated with imatinib. In this short time, research has progressed very rapidly; imatinib is now licensed in many countries for the treatment of patients with advanced CML, interferon-resistant Ph-positive leukaemias, or GISTs. The indications will soon include newly diagnosed CML. Resistance to imatinib has also been the subject of intense research and results have quickly identified the molecular nature of resistance in many, but not all, cases.

BCR-ABL probably remains crucial for leukaemic cells even at relapse; at least one general mechanism of resistance involves maintenance of an active BCR-ABL enzyme inside leukaemic cells, despite the presence of imatinib. The high frequency of *BCR-ABL* mutations and amplifications represents the high degree of heterogeneity in patients with advanced leukaemias, in whom millions of different clones probably exist.

The evidence also indicates that a single inhibitor is unlikely to be able to bind and block all the mutants described so far. Therefore, prevention of resistance will probably be better than attempts to overcome it.[61] In this context, the notion that leukaemic cells are exposed to imatinib concentrations substantially lower than those measured in plasma and the fact that some mutations show a low resistance index are important observations; these deserve experimental validation through clinical trials of substantially higher doses (administered parenterally) for a limited period, or in combination with different treatment modalities. Alternatively, imatinib analogues with higher activity and discriminatory ability between ABL and Kit/PDGFR could produce better therapeutic activity.

Several combination treatments are presently under evaluation (interferon, cytarabine, hydroxyurea); whether drugs without selective activity against leukaemic cells will be able to increase the therapeutic ratio of imatinib remains uncertain.

Search strategy and selection criteria

References for this review were identified by searches of PubMed with the key words "imatinib", "STI571", and "CGP57148B". Data were also obtained from abstracts and presentations given at the 2001 Meeting of the American Society of Hematology (December 7–11, 2001, Orlando, FL, USA). Some unpublished data were provided from our own research or from research carried out at other laboratories.

Further reading

Since the submission of this review, several new BCR-ABL mutations have been described. Additional material can be found in a database of patients resistant to imatinib at http://www.istitutotumori.mi.it/int/oncologia/proteine_ENG.asp?LinkAttivo=3

Conflict of interest

CBG-P is a coinvestigator of three registrative trials of imatinib sponsored by Novartis.

Acknowledgments

We thank Andreas Hochhaus and Susan Brandford for communicating their unpublished results and Marc Gasser for preparing the figures and useful discussion. This review was funded in part by the Italian Association for Cancer Research (AIRC), MURST COFIN 2001, Italian Ministry of Health (Ricerca Finalizzata 2001), the Josè Carreras International Foundation, and the Antonio Castelnuovo Foundation.

References

1 Goldman JM, Druker BJ. Chronic myeloid leukemia: current treatment options. *Blood* 2001; **98:** 2039–42.
2 Buchdunger E, Zimmermann J, Mett H, et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylamino-pyrimidine derivative. *Cancer Res* 1996; **56:** 100–04.
3 Zimmermann J, Buchdunger E, Mett H, et al. Potent and selective inhibitors of the Abl-kinase: phenylamino-pyrimidine (PAP) derivatives. *Bioorg Med Chem Lett* 1997; **7:** 187–92.
4 Okuda K, Weisberg E, Gilliland DG, Griffin JD. ARG tyrosine kinase activity is inhibited by STI571. *Blood* 2001; **97:** 2440–48.
5 Konopa J, Watanabe S, Witte O. An alteration of the human c-abl

For personal use. Only reproduce with permission from The Lancet Publishing Group.

protein in K562 leukemia cells unmasks associated tyrosine kinase activity. *Cell* 1984; **37:** 1035–42.
6 Pluk H, Dorey K, Superti-Furga G. Autoinhibition of c-Abl. *Cell* 2002; **108:** 247–59.
7 Kantarjian H, Melo JV, Tura S, et al. Chronic myelogenous leukemia: disease biology and current and future therapeutic strategies. *Hematology (Am Soc Hematol Educ Program)*. 2000: 90–109.
8 Mauro MJ, O'Dwyer M, Heinrich MC, Druker BJ. STI571: a paradigm of new agents for cancer therapeutics. *J Clin Oncol* 2002; **20:** 325–34.
9 Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 1996; **2:** 561–66.
10 Gambacorti-Passerini C, le Coutre P, Mologni L, et al. Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis. *Blood Cells Mol Dis* 1997; **23:** 380–94.
11 Deininger MW, Goldman JM, Lydon N, Melo JV. The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. *Blood* 1997; **90:** 3691–98.
12 le Coutre P, Mologni L, Cleris L, et al. In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. *J Natl Cancer Inst* 1999; **91:** 163–68.
13 Schindler T, Bornmann W, Pellicena P, et al. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 2000; **289:** 1938–42.
14 Ottmann OG, Druker BJ, Sawyers CL, et al. A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. *Blood* 2002; **100:** 1965–71.
15 Sawyers CL, Hochhaus A, Feldman E, et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood* 2002; **99:** 3530–39.
16 Talpaz M, Silver RT, Druker BJ, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood* 2002; **99:** 1928–37.
17 Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 2002; **346:** 645–52.
18 Druker B. STI571 (Gleevec/Glivec, imatinib) versus interferon (IFN) + cytarabine as initial therapy for patients with CML: results of a randomized study. *Proc Am Soc Clin Oncol* 2002; **20:** (abstr 1)
19 Magnusson MK, Meade KE, Nakamura R, et al. Activity of STI571 in chronic myelomonocytic leukemia with a platelet-derived growth factor beta receptor fusion oncogene. *Blood* 2002; **100:** 1088–91.
20 Apperley JF, Gardembas M, Melo JV, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N Engl J Med* 2002; **347:** 481–87.
21 van Oosterom AT, Judson I, Verweij J, et al. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* 2001; **358:** 1421–23.
22 Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Eng J Med* 2002; **347:** 472–80.
23 Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002; **20:** 1692–703.
24 Schaller JL, Burkland GA. Case report: rapid and complete control of idiopathic hypereosinophilia with imatinib mesylate. *Med Gen Med* 2001; **3:** 9.
25 Gleich GJ, Leiferman KM, Pardanani A, et al. Treatment of hypereosinophilic syndrome with imatinib mesilate. *Lancet* 2002; **359:** 1577–78.
26 Maki RG, Awan RA, Dixon RH, et al. Differential sensitivity to imatinib of 2 patients with metastatic sarcoma arising from dermatofibrosarcoma protuberans. *Int J Cancer* 2002; **100:** 623–26.
27 Rubin BP, Schuetze SM, Eary JF, et al. Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. *J Clin Oncol* 2002; **20:** 3586–91.
28 Gambacorti-Passerini C, Bungaro S, Cazzaniga G, et al. BCR-ABL gene amplification causes reversible cytogenetic relapse and resistance to imatinib (STI571) in a chronic phase CML patients. *Blood* 2002; **100:** 216a (abstr 4376).
29 Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001; **344:** 1031–37.
30 Gambacorti-Passerini C, Rossi F, Verga M, et al. Differences between in vivo and in vitro sensitivity to imatinib of Bcr/Abl+ cells obtained from leukemic patients. *Blood Cells Mol Dis* 2002; **28:** 361–72.
31 Gorre ME, Shah NP, Ellwood K, et al. Roots of clinical resistance to STI-571 cancer therapy. *Science* 2001; **293:** 2163 (abstr).
32 le Coutre P, Tassi E, Varella-Garcia M, et al. Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. *Blood* 2000; **95:** 1758–66.
33 Weisberg E, Griffin JD. Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. *Blood* 2000; **95:** 3498–505.
34 Mahon FX, Deininger MW, Schultheis B, et al. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. *Blood* 2000; **96:** 1070–79.
35 Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* 2001; **293:** 876–80.
36 Branford S, Rudzki Z, Walsh S, et al. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood* 2002; **99:** 3472–75.
37 Hofmann WK, de Vos S, Elashoff D, et al. Relation between resistance of Philadelphia-chromosome-positive acute lymphoblastic leukaemia to the tyrosine kinase inhibitor STI571 and gene-expression profiles: a gene-expression study. *Lancet* 2002; **359:** 481–86.
38 Kreil S, Muller C, Lahaye T, et al. Molecular and chromosomal mechanisms of resistance in CML patients after STI571 (Glivec) therapy. *Blood* 2001; **98:** 435a (abstr 1823).
39 Tipping AJ, Mahon FX, Lagarde V, et al. Restoration of sensitivity to STI571 in STI571-resistant chronic myeloid leukemia cells. *Blood* 2001; **98:** 3864–67.
40 Nagar B, Bornmann WG, Pellicena P, et al. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res* 2002; **62:** 4236–43.
41 von Bubnoff N, Schneller F, Peschel C, Duyster J. BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet* 2002; **359:** 487–91.
42 Shah NP, Nicoll JM, Gorre ME, et al. Resistance to Gleevec: sequence analysis reveals a spectrum of BCR/ABL kinase domain mutations in both acquired- and de novo- resistant cases of chronic myelogenous leukemia (CML) in myeloid blast crisis. *Blood* 2001; **98:** 3205 (abstr 770).
43 Barthe C, Cony-Makhoul P, Melo JV, Mahon JR. Roots of clinical resistance to STI-571 cancer therapy. *Science* 2001; **293:** 2163.
44 Hochhaus A, Kreil S, Corbin A, et al. Roots of clinical resistance to STI-571 cancer therapy. *Science* 2001; **293:** 2163.
45 Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, et al. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood* 2002; **100:** 1014–18.
46 Sawyers CL. Research on resistance to cancer drug Gleevec. *Science* 2001; **294:** 1834.
47 Hubbard SR. Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog. *Embo J* 1997; **16:** 5572–81.
48 Parang K, Till JH, Ablooglu AJ, et al. Mechanism-based design of a protein kinase inhibitor. *Nat Struct Biol* 2001; **8:** 37–41.
49 Till JH, Ablooglu AJ, Frankel M, et al. Crystallographic and solution studies of an activation loop mutant of the insulin receptor tyrosine kinase: insights into kinase mechanism. *J Biol Chem* 2001; **276:** 10049–55.
50 Muller CW, Schlauderer GJ, Reinstein J, Schulz GE. Adenylate kinase motions during catalysis: an energetic counterweight balancing substrate binding. *Structure* 1996; **4:** 147–56.

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51 Shah N, Nicoll J, Nagar B, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2002; **2:** 117.
52 Corbin AS, Buchdunger E, Pascal F, Druker BJ. Analysis of the structural basis of specificity of inhibition of the Abl kinase by STI571. *J Biol Chem* 2002; **277:** 32214–19.
53 Gambacorti-Passerini C, Zucchetti M, Russo D, et al. Alpha 1 acid glycoprotein (AGP) binds to STI571 and substantially alters its pharmacokinetics in chronic nyeloid leukemia patients. *Clin Cancer Res* 2003; **9:** in press.
54 Ford J. STI571 investigators' brochure. Basel: Novartis AG, 2001.
55 Gambacorti-Passerini C, Corneo G, D'Incalci M. Roots of clinical resistance to STI-571 cancer therapy. *Science* 2001; **293:** 2163.
56 Gambacorti-Passerini C. Decrease of resistance to imatinib in leukaemia. *Lancet* 2002; **359:** 1776–77.
57 Larghero J, Mahon F, Madalaine-Chambrin I, et al. Elevated l evels of the plasma protein alpha 1 acid glycoprotein in chronic myelogenous leukemia in blast crisis mediate pharmacological resistance to Gleevec (STI571, imatinib) in vitro and are associated with primary resistance in vivo. *Blood* 2001; **98:** (abstr 616) 2582.
58 le Coutre P, Kreuzer K, Na I, et al. Determination of -1 acid glycoprotein in patients with Ph+ chronic myeloid leukemia during the first 13 weeks of therapy with STI571. *Blood Cells Mol Dis* 2002; **28:** 75–85.
59 Gambacorti-Passerini C, Barni R, le Coutre P, et al. Role of alpha 1 acid glycoprotein in the in vivo resistance of human BCR-ABL(+) leukemic cells to the abl inhibitor STI571. *J Natl Cancer Inst* 2000; **92:** 1641–50.
60 Hofmann WK, Jones LC, Lemp NA, et al. Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR-ABL gene mutation. *Blood* 2002; **99:** 1860–62.
61 Griffin JD. Resistance to targeted therapy in leukaemia. *Lancet* 2002; **359:** 458–59.

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